BLOOD COMPOSITION OF THE COCKROACH, LEUCOPHAEA MADERAE FABRICIUS*

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The chemistry of insect haemolymph has been studied extensively since the pioneer work of Bishop, Briggs and Ronzoni (1925) on the blood of the honey bee larva, *Apis mellifica.* Florkin (1936 a, b, c) published a series of papers on protein and non-protein nitrogen, glucose and uric acid concentrations in the blood of the silkworm, *Bombyx mori* and of the water beetle, *Hydrophilus piceus* at different stages of development. Florkin (1937) described the biochemical characteristics of insect blood as (1) a high concentration of non-protein nitrogen, 50 to 80 per cent of which is amino nitrogen; (2) a high concentration of reducing substances, very little of which is glucose; (3) a high concentration of magnesium.

Florkin and Duchâteau (1942) recognizing the high amino nitrogen so characteristic for insect blood, attempted to isolate biochemically the individual amino acids. They were able to identify tyrosine and histidine in the blood of the beetle, *Dytiscus marginalis.* Pratt (1950) using the newer chromatographic methods, determined the free amino acids in the blood of the honey bee, *Apis mellifica,* the bee moth, *Galleria mellonella* and the cockroach, *Blattella germanica.* He found that the amino acids glycine, alanine, glutamic acid, tyrosine, leucine or isoleucine, methionine, proline, serine and valine were present in each species.

Florkin, Duchâteau and Leclercq (1949) separated insects into two groups depending on the concentration of sodium in the blood. Since that time many studies on the inorganic ion content have been made, the results of which have been compiled into a table by Buck (1953).

Most of the published material concerns those insects with a

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holometabolus life cycle. In these forms the amino nitrogen was approximately 200 mg. per cent which is about 50 to 80 per cent of the total non-protein nitrogen. Todd (1957) studied the organic constituents in the blood of a paurometabolous insect, the American cockroach, *Periplaneta americana* and found total amino nitrogen to average only 78 mg. per cent which was 40 per cent of the non-protein nitrogen. Because of the paucity of work on paurometabolous insects the present work was undertaken to determine the concentration of various inorganic ions, nitrogenous compounds including individual amino acids, and reducing substances in the blood of another paurometabolous insect. The tropical cockroach, *Leucophaea maderae,* was chosen because of its large size and the ease with which its blood can be obtained.

MATERIAL AND METHODS

The cockroaches were kept at room temperature in glass jars, and were supplied with laboratory food pellets and water. The insects were etherized to obtain uncoagulated blood (Ludwig 1951). The antennae were clipped with scissors and the haemolymph allowed to drip into the depression of a porcelain spot-plate. One-tenth of a milliliter of blood was used for each test.

The ionorganic ions calcium, potassium and sodium were measured by the Beckman flame photometer, and magnesium was determined by the method of Denis as modified by Fiske and Subbarow (1925). The concentration of reducing substances was obtained by the Hagedorn and Jensen technique as outlined in Hawk, Oser and Summerson (1951). Trehalose was determined using the anthrone test (Wyatt and Kalf 1957). Protein and non-protein nitrogen were determined by the micro-Kjeldahl procedure. Urea nitrogen was determined according to the method of Ormsby (1942) as modified by Kawerau (1946). Uric acid nitrogen was measured by the method of Brown (1945), and amino acid nitrogen by that of Danielson as modified by Frame, Russell and Wilhelmi (1943).

Amino acids were separated qualitatively by the method of McFarren (1951) for paper partition chromatography, and the quantitative determinations were made according to the techniques described by McFarren, Brand and Rutkowski (1951) and McFarren and Mills (1952). The filtrate for these determina-

tions was prepared according to the procedure outlined by Pratt (1950).

OBSERVATIONS

The results of the determinations on inorganic ions and organic compounds are shown in table 1. The concentration of inorganic

TABLE 1. CONCENTRATION OF CERTAIN ORGANIC AND INORGANIC COMPOUNDS IN THE HAEMOLYMPH.

Substance	No. of tests	Mg. per cent		
		minimum	maximum	average
Sodium	5	236	262	230
Potassium	5	29	55	38
Calcium	5	13	20	16.5
Magnesium	5	4.2	4.7	4.6
Protein nitrogen	10	532.38	812.58	686.46
Non-protein nitrogen	10	126.09	448.32	235.36
Urea nitrogen	10	7.50	9.10	7.97
Urea	10	16.00	19.50	17.05
Uric acid nitrogen	12	3.22	5.18	4.08
Uric acid	12	9.67	15.55	12.24
Amino acid nitrogen	10	76.15	97.27	85.44
Reducing compounds	12	136.00	340.00	228.00
Non-fermentable reducing compounds	10	56.00	320.00	163.00
Fermentable material (as glucose)	—	—	—	65.00
Trehalose	7	580.00	780.00	677.00

ions were calcium 16.5, potassium 38.0, sodium 230 and magnesium 4.2 mg. per cent. Total reducing compounds were 228, non-fermentable reducing compounds 163, and fermentable material (glucose) 65 mg. per cent. Trehalose a non-reducing disaccharide was 677 mg. per cent. Concentrations of nitrogen in various compounds were protein 686.46 and non-protein nitrogen 235.36. Fractionation of the non-protein nitrogen showed urea 7.9, uric acid 4.08 and amino acid nitrogen to be 85.44 mg. per cent.

The amino acids separated and identified in four solvent systems are shown in table 2. Normal-butanol-acetic acid-water was the best of all the systems used for the identification of the individual amino acids because it required no adjustment of pH

TABLE 2. AMINO ACIDS IDENTIFIED BY PAPER PARTITION CHROMATOGRAPHY.

Solvent	pH	Time for runs	Amino acids
Normal-butanol-acetic acid-water		51–55 hours	alanine arginine cysteine glycine histidine hydroxyproline norleucine ornithine phenylalanine proline serine threonine tyrosine valine
ortho-cresol	8.4	51–55 hours	beta alanine citrulline glycine methionine norleucine ornithine taurine threonine valine
Phenol	12.0	36–40 hours	alanine aspartic acid glutamic acid glycine serine threonine
Phenol	6.2	17–20 hours	Composite spots 1. norleucine phenylalanine tryptophane valine 2. alanine tyrosine 3. arginine histidine threonine 4. cysteine ornithine serine

nor previous buffering of the paper. The resolution and separation of spots of both the unknown in the filtrate and the standards were the clearest in this solvent. Fourteen amino acids were identified with n-butanol-acetice acid, the most in any solvent used. Ortho-cresol, saturated with buffer at pH 8.4, yielded the identification of nine amino acids but only four of these were different from those identified with n-butanol-acetic acid. They were methionine, beta alanine, taurine and citrulline. Phenol at pH 12.0 aided in the separation of six amino acids but again four had been previously identified with n-butanol-acetic acid. The two which had not been identified in the other solvents were aspartic and glutamic acids. Phenol at pH 6.2 was not a satisfactory solvent because the amino acids did not separate into individual spots. Composite spots were obtained and therefore, no positive identification could be made with this solvent.

Table 3 lists the quantitative results of the individual amino

TABLE 3. QUANTITATIVE MEASUREMENTS OF AMINO ACIDS ISOLATED BY PAPER CHROMATOGRAPHY.

Figures in parenthesis indicate number of readings made in each solvent.

Amino acid	Solvent		Amino nitrogen mg. per cent
Alanine	n-butanol-acetic acid	(2)	6.20
Arginine	n-butanol-acetic acid	(3)	13.16
Aspartic acid	phenol pH 12.0	(2)	trace
Beta alanine	o-cresol pH 8.4	(3)	trace
Citrulline	o-cresol pH 8.4	(3)	8.06
Cysteine	n-butanol-acetic acid	(2)	trace
Glycine	phenol pH 12.00	(2)	6.62
	o-cresol 8.40	(3)	
Histidine	n-butanol-acetic acid	(2)	5.05
Methionine	o-cresol pH 8.4	(3)	3.60
Norleucine	n-butanol-acetic acid	(3)	13.16
Ornithine	n-butanol-acetic acid	(2)	5.30
Phenylalanine	n-butanol-acetic acid	(2)	5.50
Proline	n-butanol-acetic acid	(2)	trace
Serine	phenol pH 12.0	(2)	2.60
Taurine	o-cresol pH 8.4	(3)	trace
Threonine	n-butanol-acetic acid	(3)	2.48
	phenol 12.0	(4)	
Tyrosine	n-butanol-acetic acid	(2)	2.55
Valine	n-butanol-acetic acid	(2)	3.60
Total			92.66

acids which were determined using the same solvent systems as for the qualitative results. However, in some instances not all the amino acids isolated in a particular solvent could be measured quantitatively because they were not present in sufficient concentrations. They are recorded as trace amounts. The amino nitrogen for each amino acid obtained in each solvent was averaged for the final figure. The total amino nitrogen determined by this method was 92.66 mg per cent.

DISCUSSION

The blood of the cockroach, *Leucophaea maderae* has a high sodium and low potassium as well as a low magnesium of 4.2 mg. per cent. Thus by its ion index this insect falls into the category of high sodium described by Florkin, Duchâteau and Leclercq (1949). They described insects with a high sodium as being ancestral forms or those whose development was independent of the evolution of plants. It is well known that the cockroach is a primitive insect. Barsa (1954) studied the concentrations of inorganic ions in the blood of an insect representative of the two groups. She found that the grasshopper, *Chortophaga viridifasciata,* another primitive insect, had a high sodium and low potassium and the pupa of the Cynthia moth *Samia walkeri* a high potassium and low sodium but both insects had a high magnesium index. However, in spite of the high magnesium concentration in the blood of insects, this ion is toxic to the insect. It was suggested that magnesium is not free but in the bound form. Therefore, it would seem that these insects do not conform to the rule of Florkin, Duchâteau and Leclercq (1949) that those insects with a low sodium and high potassium index have a higher magnesium content than those of the more primitive group. However, the grasshopper, which is the exception with both a high sodium and magnesium, is not as primitive as the cockroach.

The concentration of reducing substances is expressed as equivalents of glucose although only about one-fourth of the total reducing substances is glucose. Many other substances such as ascorbic acid, uric acid and glutathione will reduce ferricyanide in hot alkaline solution. A characteristic of insect haemolymph is its high concentration of non-fermentable reducing substances. In *L. maderae* they amount to 163 mg. per

cent. The exact nature of these compounds is still unknown. Buck (1953) suggested that they are a complex of phenolic compounds concerned with the hardening and darkening of the cuticle. It has been shown that these polyphenols and phenolic amino acids have a high reducing value.

Wyatt, Loughheed and Wyatt (1956) in their work on the chemistry of the silkworm, *Bombyx mori,* noticed that acid hydrolysis of the haemolymph caused the release of a fermentable substance. Wyatt and Kalf (1957) identified this substance as trehalose, a non-reducing disaccharide. They reported that in 10 insects this substance ranged between 306 in *Bombyx* larvae, to 1,398 mg. per cent in the moth, *Telea polyphemus.* In the cockroach the concentration of this sugar is 677 mg. per cent. Trehalose thus appears to be a major blood sugar in this species.

The concentrations of the nitrogenous substances in the blood of *L. maderae* are in agreement with those found in other insects as shown in the tables compiled by Buck (1953). In the tropical cockroach, amino nitrogen is 85 mg. per cent or about 35 per cent of the total non-protein nitrogen. These results are in agreement with those of Todd (1957) on the blood of the American cockroach, *Periplaneta americana.* These values are lower than the 50 to 80 per cent quoted by Buck (1953). However, he based that percentage on insects with a holometabolous life cycle.

Po-Chedley (1956) isolated 21 amino acids from the blood of the oriental beetle, *Anomala orientalis.* In phenol at pH 12.0, he identified nine amino acids. In the present work, six were identified in this solvent. Po-Chedley was able to separate leucine and isoleucine with collidine. However, this solvent did not completely separate the amino acids in the blood filtrate of the cockroach and was discarded early in the experiments. Po-Chedley was able to isolate lysine and taurine with n-butanol-acetic acid. However, in the present work lysine could not be positively identified. Taurine was isolated in o-cresol pH 8.4 but not in any other solvent. Pratt (1950) isolated 17 amino acids from the blood of the cockroach, *Blattella germanica* and 11 from that of the American cockroach. In *Blattella* he found no histidine, hydroxyproline, phenylalanine, tryptophane or taurine. All these were found in the blood filtrate of *L. maderae* and citrulline was identified in o-cresol at pH 8.4. To the

author's knowledge, this is the first time that this amino acid has been found in insect blood.

SUMMARY

The average concentration of inorganic ions, expressed as mg. per cent, are sodium 230, potassium 38, calcium 16.5 and magnesium 4.2.

The average concentration of the nitrogenous fractions expressed as mg. per cent, are protein 686.46 and non-protein 235.36. Fractionation of the non-protein nitrogen showed urea to average 7.97, uric acid 4.08 and amino acid nitrogen 85.44 mg. per cent.

The average concentration of reducing substances was 228, and that of non-fermentable reducing substances 163 mg. per cent. Glucose, the fermentable reducing substances averaged 65 mg. per cent. Trehalose, a non-reducing disaccharide, was 677 mg. per cent.

The nineteen amino compounds isolated by paper chromatography were alanine, arginine, aspartic acid, beta alanine, citrulline, cysteine, glycine, histidine, hydroxyproline, methionine, norleucine, ornithine, phenylalanine, proline, serine, taurine, threonine, tyrosine and valine.

The total amino nitrogen determined by paper chromatography was 92.66 mg. per cent.

The best solvent for these experiments, isolating fourteen amino acids, was n-butanol-acetic acid-water.

BIBLIOGRAPHY

BARSA, M. C. 1954. The behavior of isolated hearts of the grasshopper, *Chortophaga viridifasciata* and the moth, *Samia walkeri*, in solutions with different concentrations of sodium, potassium, calcium and magnesium. Jour. Gen. Physiol. **38**: 79–92.

BISHOP, G. H., A. P. BRIGGS AND E. RONZONI. 1925. Body fluids of the honey bee larva. II Chemical constituents of the blood, and their osmotic effects. Jour. Biol. Chem. **66**: 77–88.

BROWN, H. 1945. The determination of uric acid in human blood. Biochem. Jour. **32**: 895–902.

BUCK, J. 1953. Physical properties and chemical composition of insect blood. In: Insect Physiology. New York. (chapter 6)

FISKE, C. H. AND Y. SUBBAROW. 1925. The colorimetric determination of phosphorous. Jour. Biol. Chem. **66**: 375–400.

FLORKIN, M. 1936a. Sur la tenure en proteines du plasma sanguin des insectes. Comp. rend. Soc. belge biol. **123**: 1024.

FLORKIN, M. 1936b. Sur la glycemie plasmatique vraie des insectes. Comp. rend. Soc. belge. biol. **123**: 1249.

FLORKIN, M. 1936c. Sur le taux de l'uricemie chez les insectes. Comp. rend. Soc. belge de biol. **123**: 1247.

FLORKIN, M. 1937. Contributions a l'etude du plasma sanguin des insectes. Mem. l'Acad. roy. (Classe des Sciences) Belgique. **16**: 1–69.

FLORKIN, M. AND G. DUCHÂTEAU. 1942. Sur les acides amines du plasma sanguin des insectes. Bull. classe des. sci. Acad. de Belgique. **28**: 373–376.

FLORKIN, M., G. DUCHÂTEAU AND J. LECLERCQ. 1949. Sur les constituents inorganiques du plasma sanguin des insectes. Arch. internat. physiol. **57**: 209–210.

FRAME, E. G., J. A. RUSSELL AND A. E. WILHELMI. 1943. The colorimetric estimation of amino nitrogen in blood. Jour. Biol. Chem. **149**: 255–270.

HAWK, P. B., B. L. OSER AND W. H. SUMMERSON. 1954. Practical Physiological Chemistry. Thirteenth ed. New York.

KAWERAU, E. 1946. Biochemical analysis with the Speeker absorptiometer: 1. Estimation of urea, citrulline, allantoin and related caramido compounds. Sci. Proc. Royal Dublin Soc. **24**: 63.

LUDWIG, D. 1951. Composition of the blood of the Japanese beetle (*Popillia japonica* Newman) larvae. Physiol. Zool. **24**: 336–346.

MCFARREN, E. F. 1951. Buffered filter paper chromatography of the amino acids. Anal. Chem. **23**: 168–174.

MCFARREN, E. F. AND J. MILLS. 1952. Quantitative determinations of amino acids on filter paper chromatograms by direct photometry. Anal. Chem. **24**: 650–653.

MCFARREN, E. F., K. BRAND AND H. RUTKOWSKI. 1951. Quantitative determinations of sugars on filter paper chromatograms by direct photometry. Anal. Chem. **23**: 1146–1149.

ORMSBY, A. A. 1942. A direct colorimetric method for the determination of urea in blood and urine. Jour. Biol. Chem. **146**: 595–604.

PO-CHEDLEY, D. S. 1956. Effects of starvation on amino nitrogen in the blood of the Oriental beetle (*Anomala orientalis* Waterhouse) larvae as determined by paper partition chromatography. Ph.D. Dissertation, Fordham University.

PRATT, J. J. 1950. A qualitative analysis of the free amino acids in insect blood. Ann. Ent. Soc. America. **43**: 573–580.

TODD, M. E. 1957. The concentration of certain organic constituents in the haemolymph of the American cockroach, *Periplaneta americana* Linnaeus. Jour. N. Y. Ent. Soc. **65**: 85–88.

WYATT, G. R. AND G. F. KALF. 1957. The chemistry of insect haemolymph. II Trehalose and other carbohydrates. Jour. Gen. Physiol. **40**: 833–847.

WYATT, G. R., T. C. LOUGHHEED AND S. S. WYATT. 1956. The chemistry of insect haemolymph of the silkworm, *Bombyx mori* and two other species. Jour. Gen. Physiol. **39**: 853–868.